Some very hard problems in nature (biology-biochemistry) "solved" using physical algorithms that reduce the hardness

"Problems"	*"Algorithms"*
Search optimization	Cooperativity
Hill climbing—energy reduction	Heterogeneity
Allocation of resources	Stochasticity
Self assembly	
Reversible computation	
Satisfiability	
Controllers for nanomachines	

add your favorite problem

PENN HUNT PROJECT
September 18, 2008

Harvey Rubin MD, PhD
University of Pennsylvania

Report Documentation Page

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Cooperativity at the monomolecular level binding of B or C to the common partner A affects binding of the other

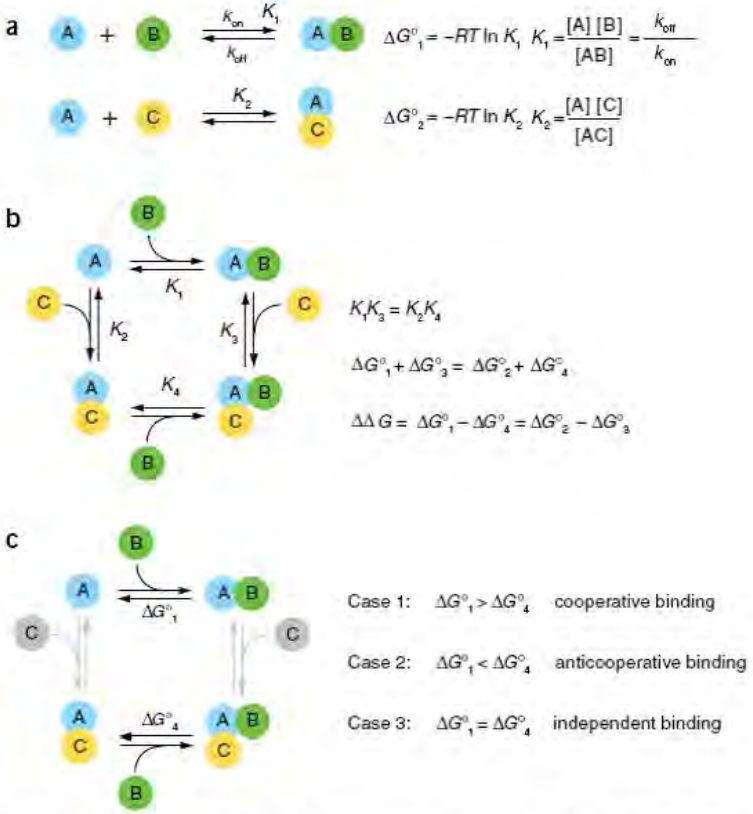
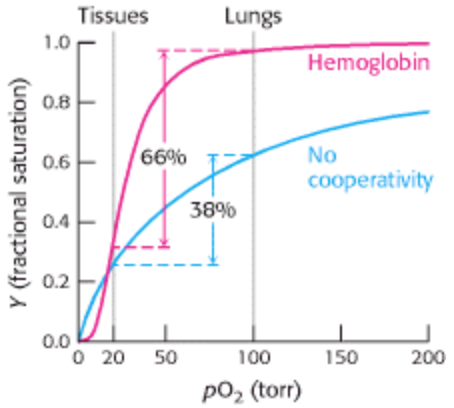


Figure 1 Thermodynamic cycles and cooperativity. (**a**) Hypothetical set of bimolecular complexes between component A and two other components (B and C), with the rate constants, equilibrium constants and free energies for complex formation. (**b**) A thermodynamic cycle for formation of the ternary complex ABC by two different possible routes: either B binds first, or C binds first. There are four equilibrium constants that describe the formation of the various complexes. Because they converge on the common product ABC, the thermodynamics must be independent of the pathway chosen around the cycle, and constraints are placed on the relative values of the equilibrium constants and hence the free energies. The thermodynamic coupling free energy ($\Delta\Delta G$) gives the difference between binding of one component in the presence of the other. (**c**) Definition of cooperativity in terms of binding of B in the presence or absence of C. The two vertical binding reactions are gray to emphasize the comparison of ΔG°_1 and ΔG°_4. If B binds better in the presence of C, the binding is cooperative. If B binds worse in the presence of C, the binding is anticooperative. In the third case, binding of B is independent of C, and there is no cooperativity.

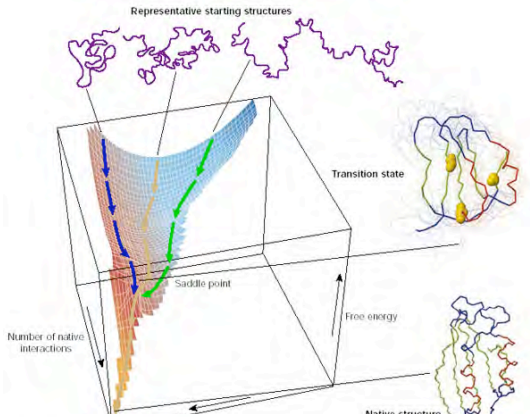
Cooperativity in macromolecular assembly
James R Williamson
volume 4 number 8 August 2008 nature chemical biology

Cooperativity/heterogeneity

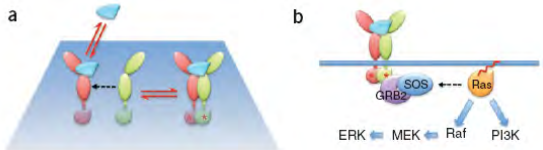
1. complex interactions among identical ligands binding to multiple sites on an oligomeric protein--oxygen binding to hemoglobin.
Homotropic *allosteric* regulators—e.g. O2
Heterotropic *allosteric* regulators—e.g. 2,3 BPG

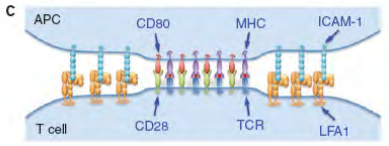


2. the thermodynamics of macromolecular conformational transitions--protein folding or nucleic acid helix-coil transitions.



3. the thermodynamics of forming multicomponent complexes—multimeric complexes, surface interactions, cellular communication, organism organization, multicellular dynamics, social structures





Cooperativity and biological complexity
Adrian Whitty nature chemical biology volume 4 number 8 august 2008

Proc. Nat. Acad. Sci. USA
Vol. 72, No. 6, pp. 2465–2467, June 1975

Interaction of Hemoglobin with Three Ligands: Organic Phosphates and the Bohr Effect

(Haldane coefficient/2,3-diphosphoglycerate/linkage equations/allosteric effect/oxygen binding)

RUTH E. BENESCH AND HARVEY RUBIN

Department of Biochemistry, Columbia University, College of Physicians & Surgeons, New York, N.Y.

Communicated by Harden M. McConnell, April 15, 1975

ABSTRACT **The assumption that the Bohr coefficient $(\Delta \log p_{50})/(\Delta pH)$ is equal to the Haldane coefficient (ΔH^+) of hemoglobin is shown to be incorrect in the presence of allosteric effectors such as 2,3-diphosphoglycerate. The theoretical relation between the two coefficients in the presence of 2,3-diphosphoglycerate is derived. Experimental data on the variation of both coefficients with diphosphoglycerate concentration are presented and shown to be in agreement with prediction.**

Therefore, the liberation of diphosphoglycerate on oxygenation must lead to an increase in its activity with increasing oxygenation, and Eqs. 1 and 2 can no longer apply.

It is the purpose of this paper to examine the relation between the Bohr and Haldane effects in the presence of 2,3-diphosphoglycerate.

RESULTS AND DISCUSSION

Box 1 A timeline showing evolution of allostery as a concept

The timeline (Fig. 1) includes some of the key experiments and realizations of the field and some insight into how the mentality of the field has shifted.

1903—The Bohr effect (sigmoidal binding curve of hemoglobin to O_2 was observed).

1910—A. Hill formulates the Hill equation to describe the sigmoidal binding of O_2 to hemoglobin.

1958—First X-ray structure (sperm whale myoglobin) solved by M. Perutz and Sir J. Cowdery Kendrew[78].

1950s—Repression of gene expression, covalent modification of enzyme activity, and feedback inhibition of enzymes are discovered[79].

1963—J. Monod renames regulatory sites 'allosteric sites.'

1965—J. Monod, J. Wyman and J.-P. Changeux propose a theoretical model of concerted allosteric transitions (MWC model)[1].

1966—D. Koshland, G. Nemethy and R. Filmer propose the sequential model for allosteric transitions (KNF model)[80].

1984—Allosteric regulation in the absence of conformational change is proposed[12].

1980s—Protein folding studies lead to the concept that proteins exist in different conformations in an "energy landscape"[81].

1990s—Mutations, covalent modifications and changes in conditions such as pH are included as allosteric effectors.

1999—Allosteric networks in the PDZ domain proposed by R. Ranganathan[50].

2006—Negative allostericity reported in the absence of conformational changes[64].

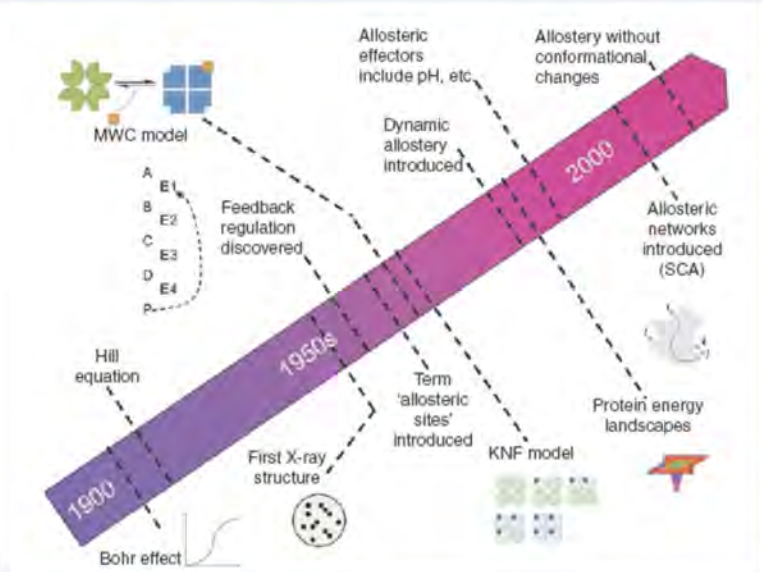
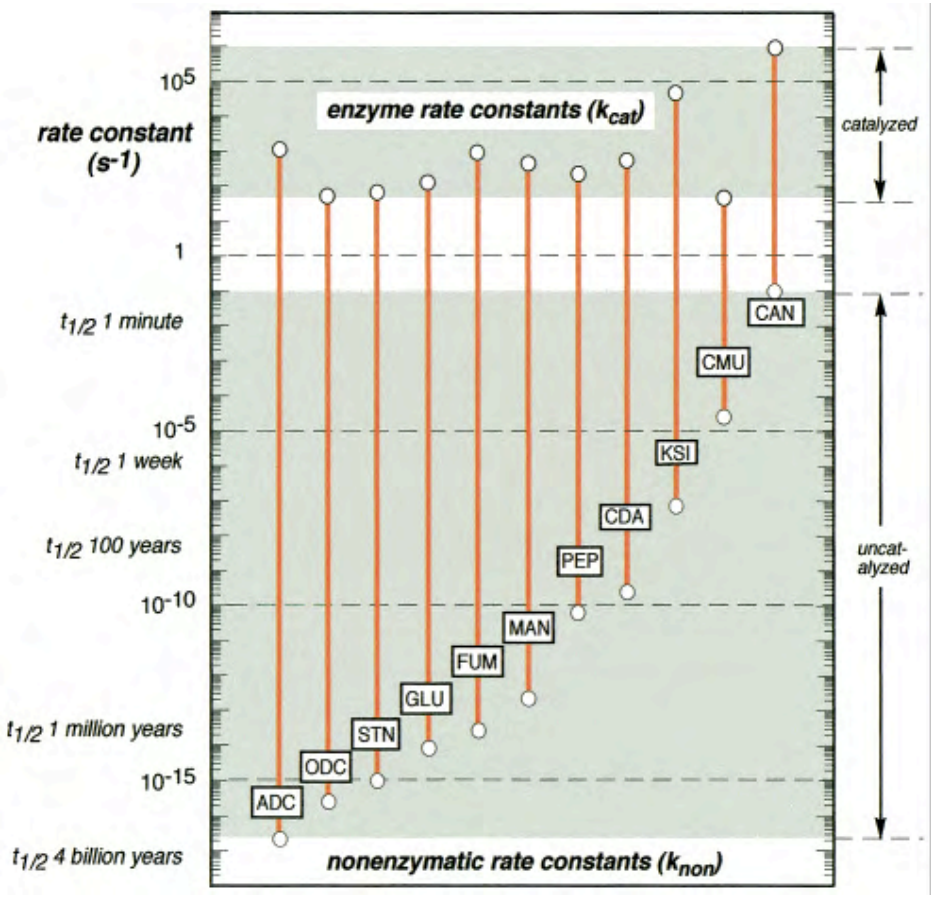


Figure 1 Timeline showing the discovery and progression of the concept of allosteric regulation in proteins.

Logarithmic scale of k_{cat} and k_{non} values for representative reactions at 25 °C. The length of each vertical bar represents the rate enhancement by each enzyme



ADC) arginine decarboxylase;
ODC) orotidine 5'-phosphate decarboxylase;
STN) staphylococcal nuclease;
GLU) sweet potato *â*-amylase; FUM) fumarase;
MAN) mandelate racemase;
PEP) carboxypeptidase B;
CDA) E. coli cytidine deaminase;
KSI) ketosteroid isomerase;
CMU) chorismate mutase;
CAN) carbonic anhydrase.

The Depth of Chemical Time and the Power of Enzymes as Catalysts
R. WOLFENDEN AND M.J. SNIDER
Acc. Chem. Res. **2001,** 34, 938-945

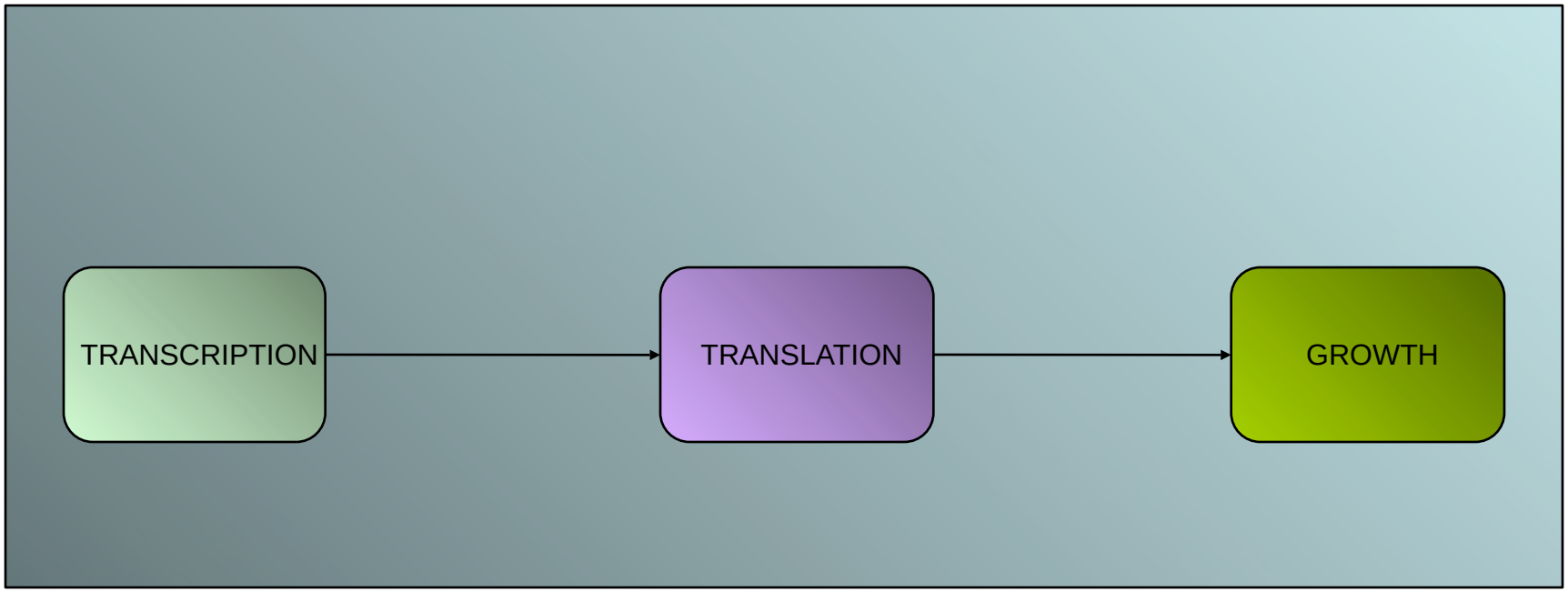
How does “Biology” cope?

Mutually Assured Destruction: Cold War exhibit at the Smithsonian

Stringent response and growth control

- Triggered by adverse conditions, e.g. starvation

- Transcription control (p)ppGpp:
  - Lack of nutrients
  - Stalled ribosomes
  - ppGpp synthesis
  - Reprogramming of transcription
- Translation shutdown
  - Proteases
  - (p)ppGpp involved
  - Activation of toxin-antitoxin modules
  - Toxin reversibly disables ribosomes



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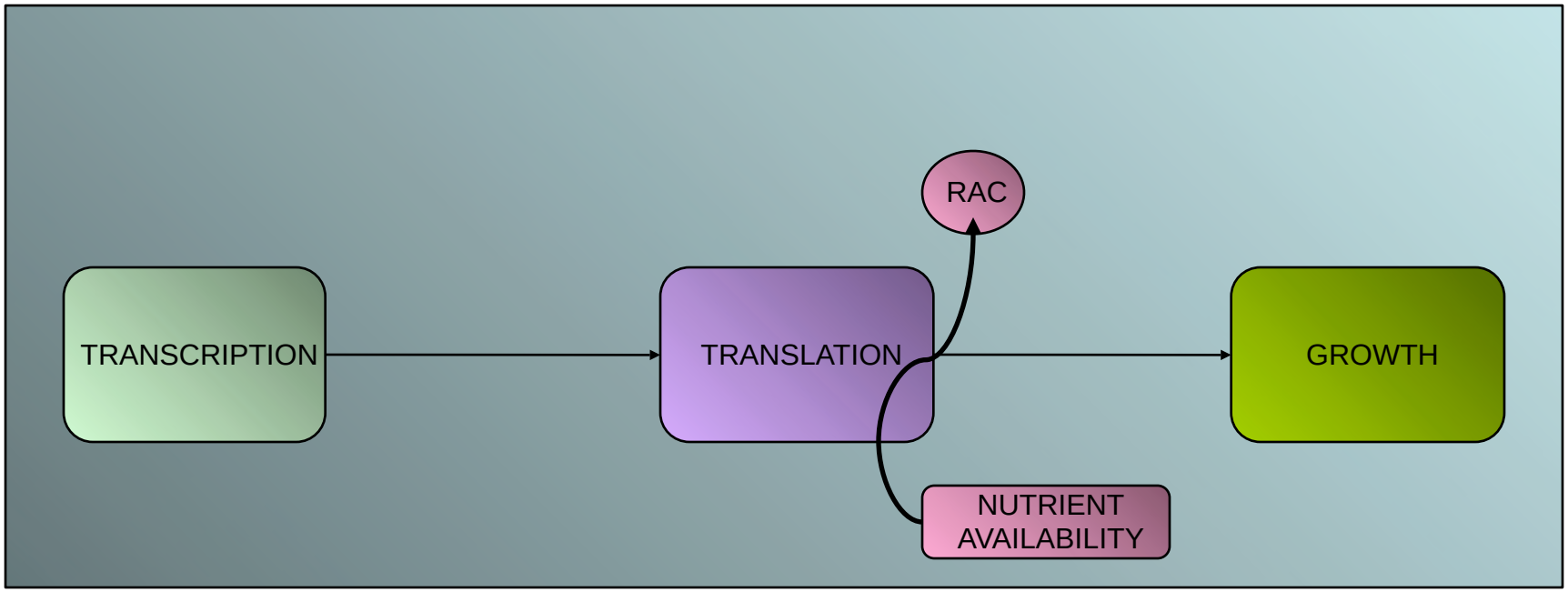

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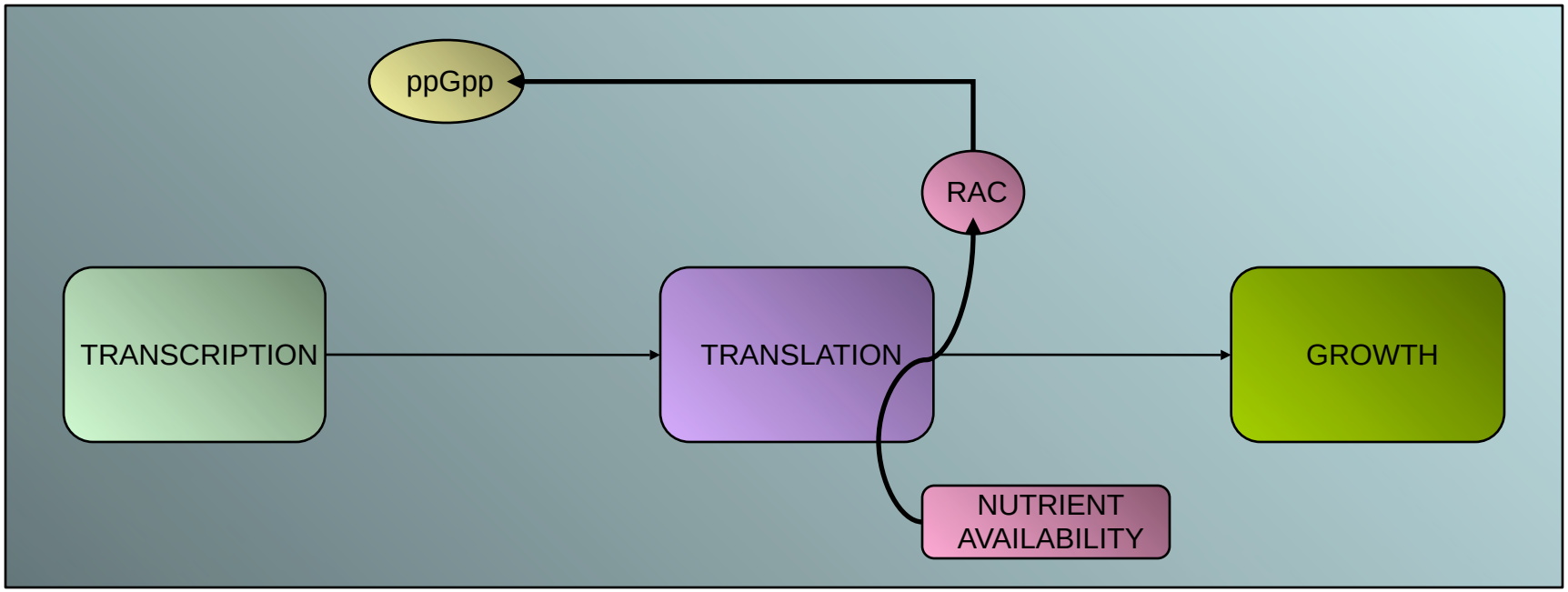

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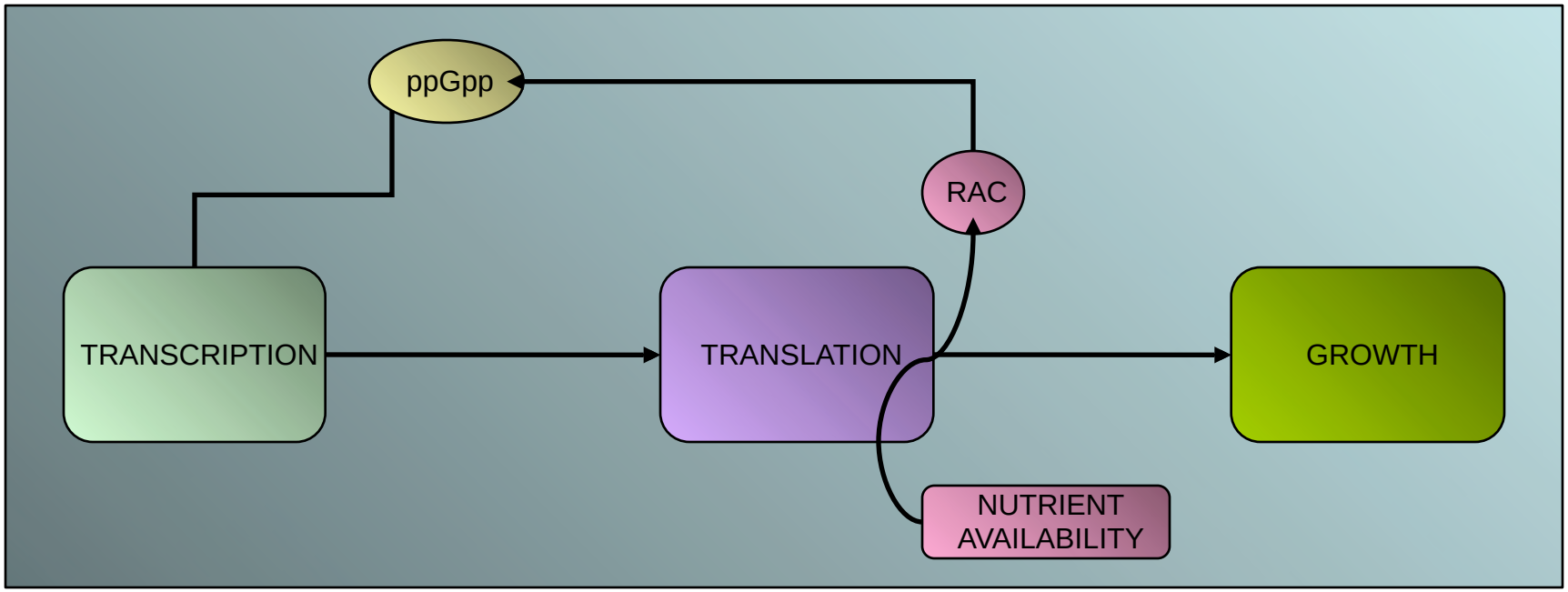

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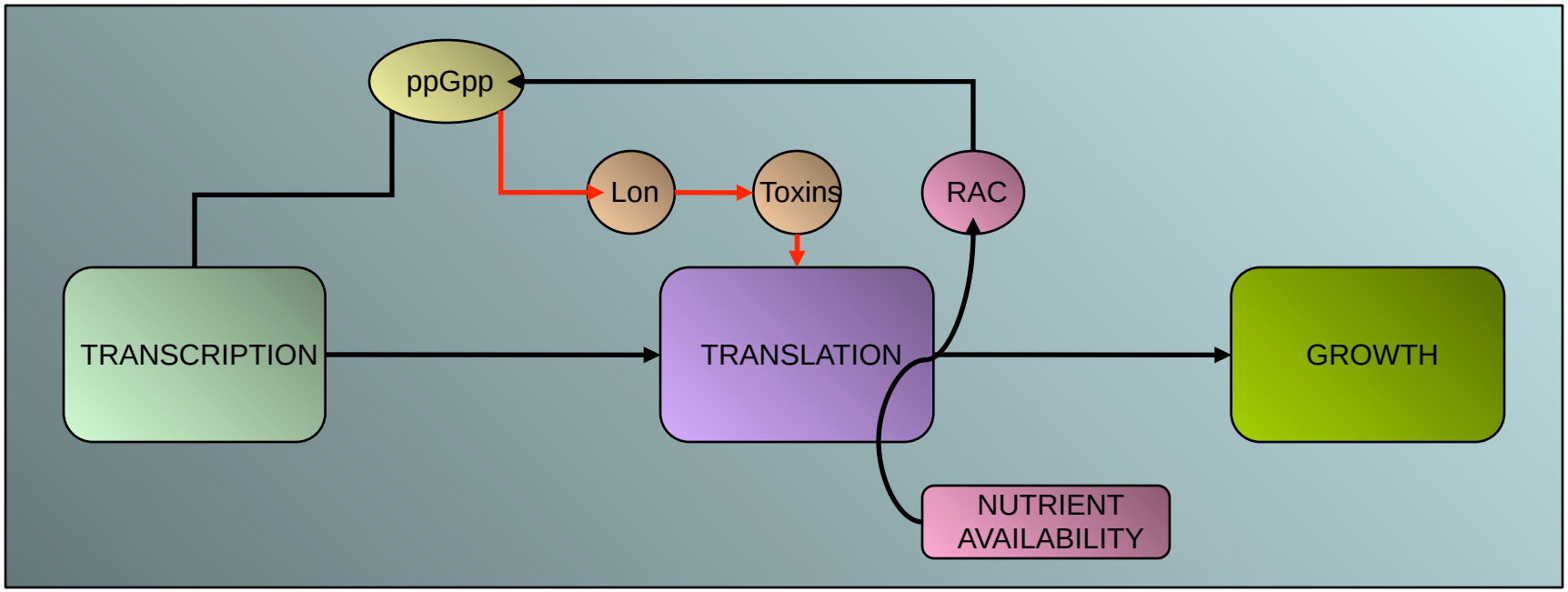
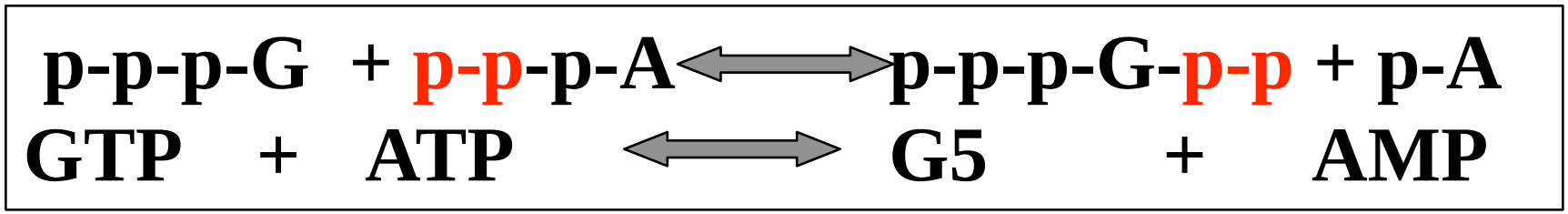
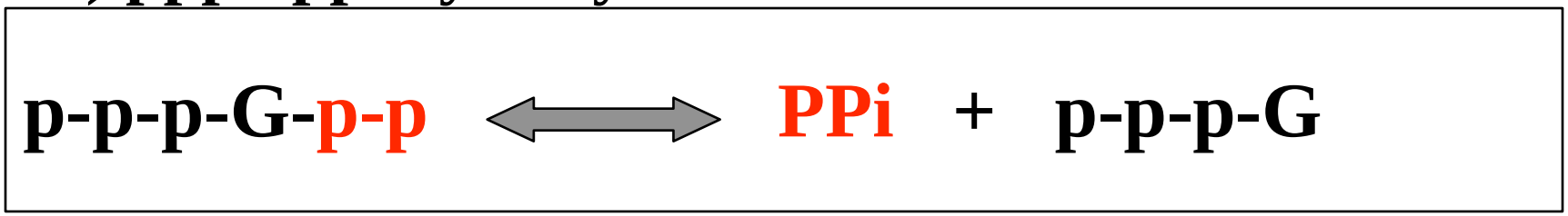


The Stringent Response is mediated by two <u>opposing</u> Rel_{Mtb} activities which must be tightly regulated

1) pppGpp synthesis:



2) pppGpp hydrolysis:

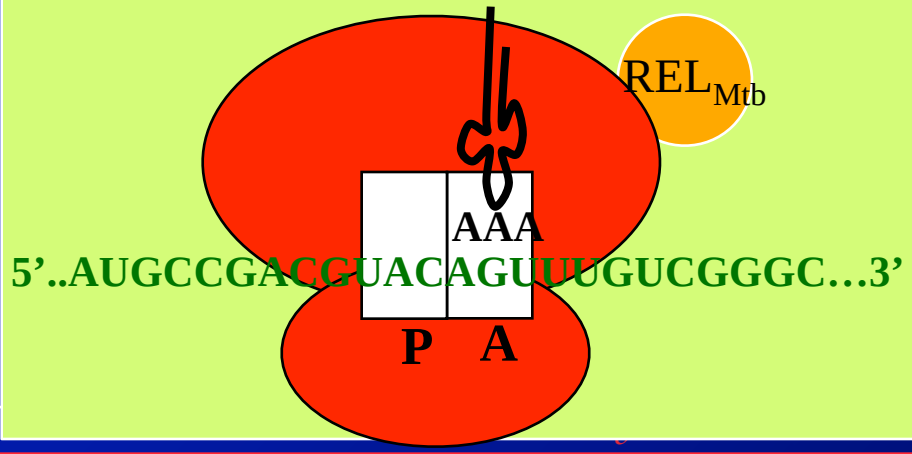


pppGpp alters RNAP kinetics and mediates the transcriptional response to environmental conditions to which Mtb is exposed

The RAC Allosterically Activates Transferase Activity

	K_{ATP} (mM)	K_{GTP} (mM)	k_{cat} (s^{-1})	k_{cat}/K_{ATP} ($mM^{-1}\ s^{-1}$)	k_{cat}/K_{GTP} ($mM^{-1}\ s^{-1}$)
Rel_{Mtb} (Basal Level)	**2.0**	**1.4**	**1.2**	**0.6**	**0.9**
Rel_{Mtb} + Ribosome•UtRNA•mRNA	**0.5**	**0.3**	**24.7**	**54.8**	**79.6**

RAC = $\underline{Rel}_{Mtb}$ $\underline{A}$ctivating $\underline{C}$omplex
Ribosome•Uncharged tRNA•mRNA



Heterogeneity even within a single molecule

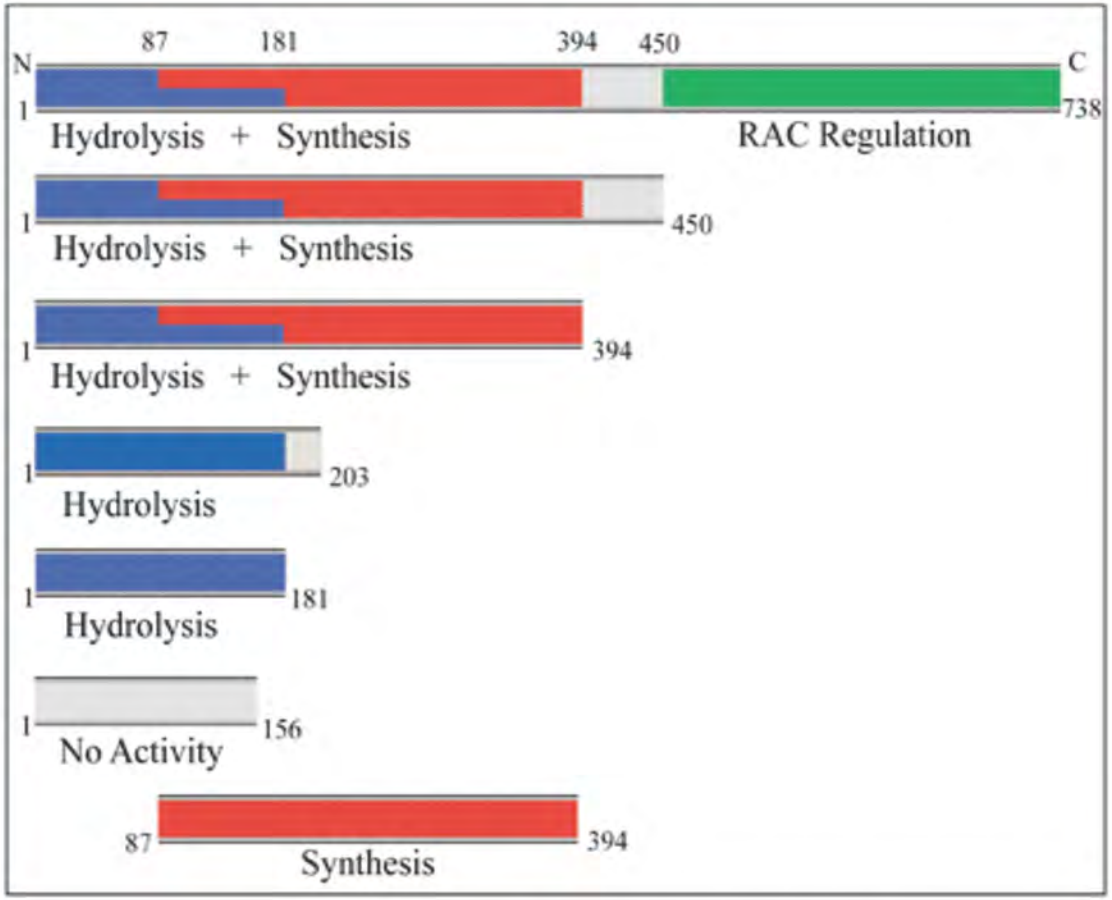
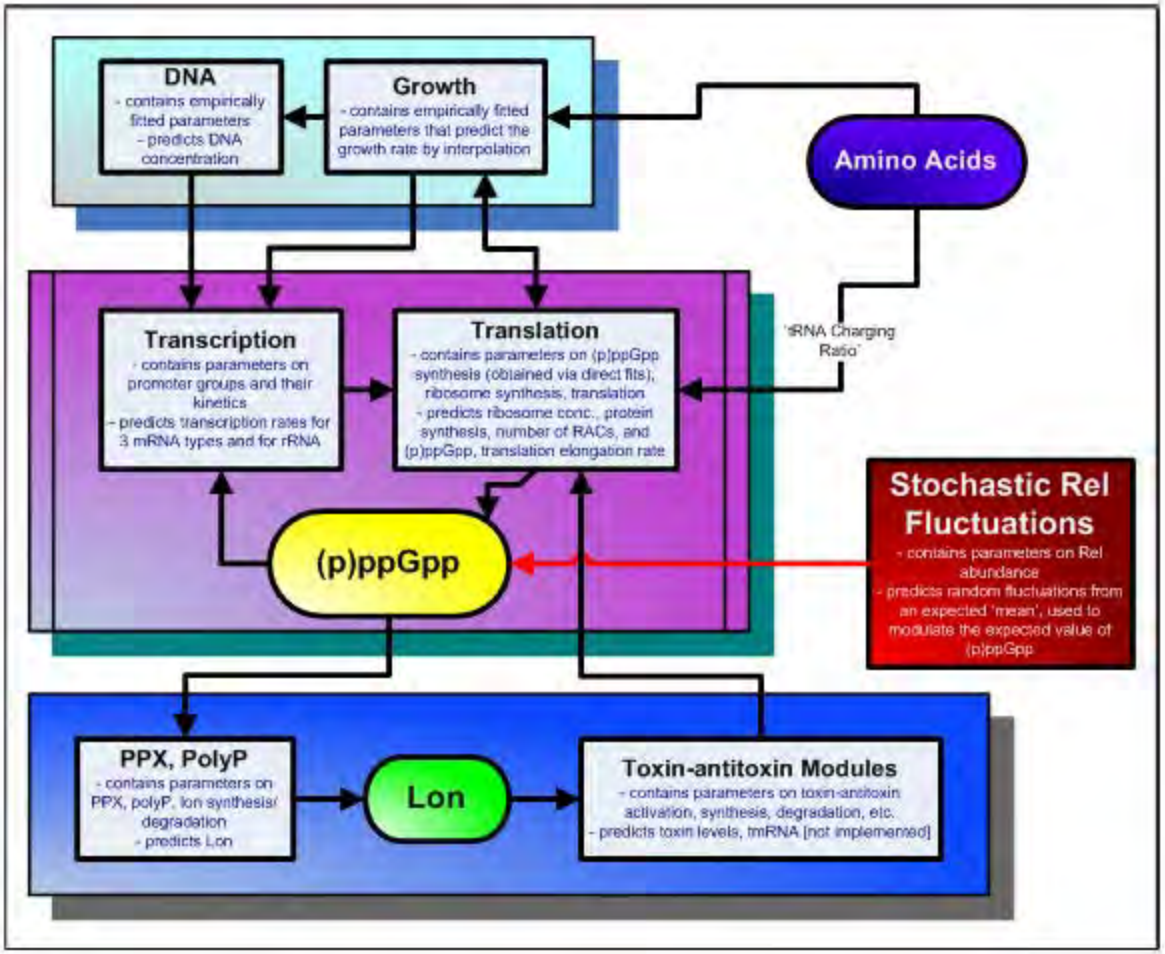
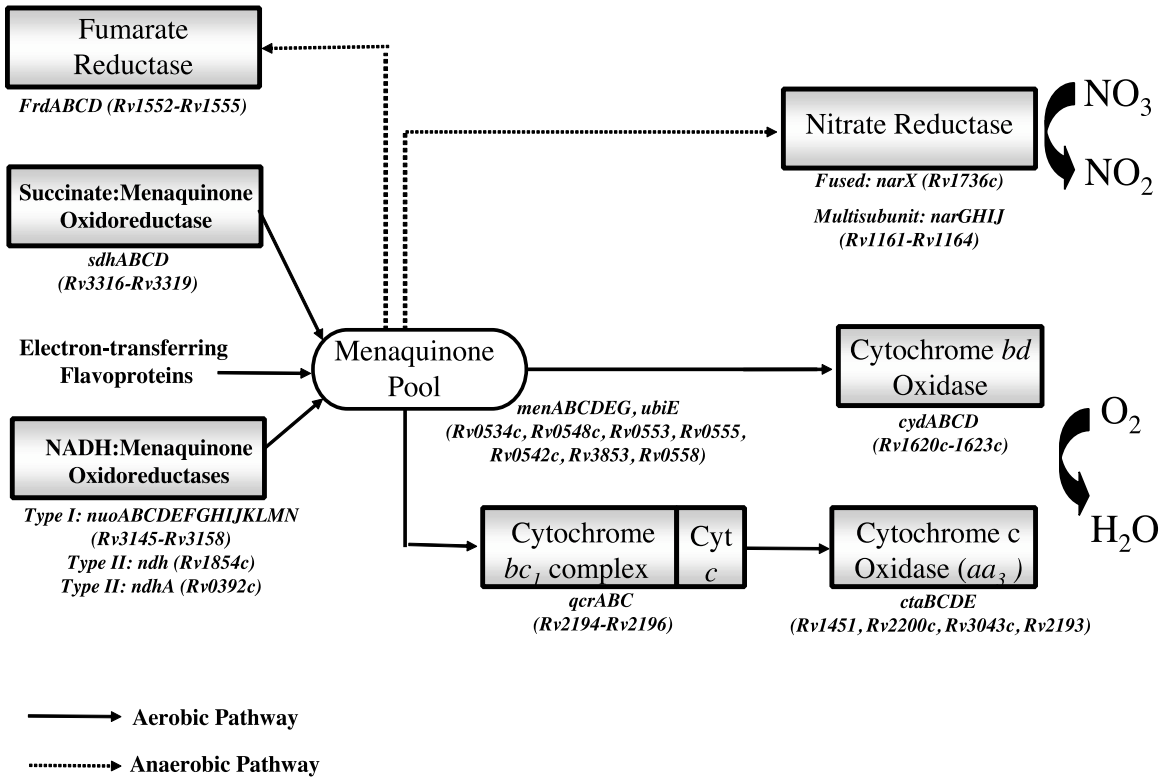


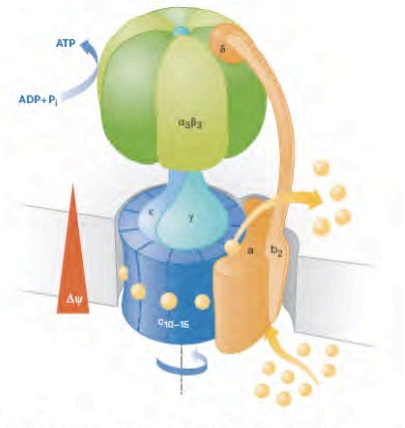
Figure 1: Summary of Rel_{Mtb} truncated. Full-lenght Rel_{Mtb} protein is at the top followed by the different truncated proteins. Animo acid numbers are at the beginning and end of each fragment and corresponding activity is listed below. 87-187 overlapping site is noted in the full-length Rel_{Mtb}

Cooperativity, hetergeneity, stochasticity



Another example: Controllers for nanomachines
Aerobic and anaerobic respiratory chain in Mtb

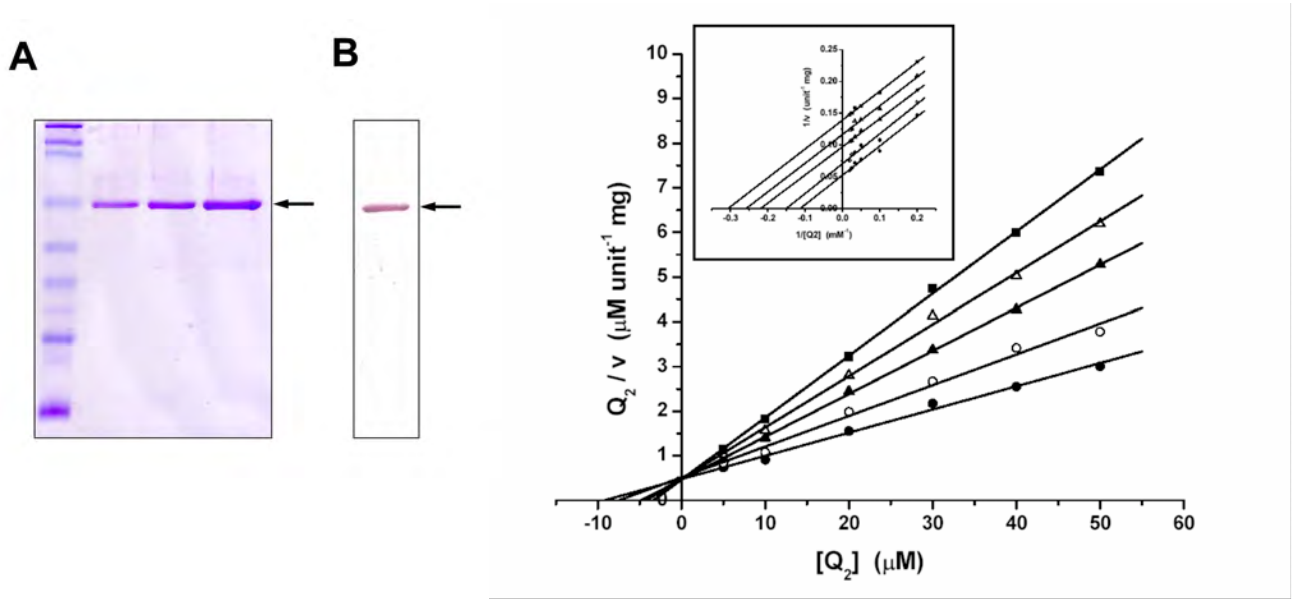




ATP synthase

Electrons enter the chain through NADH oxidoreductase

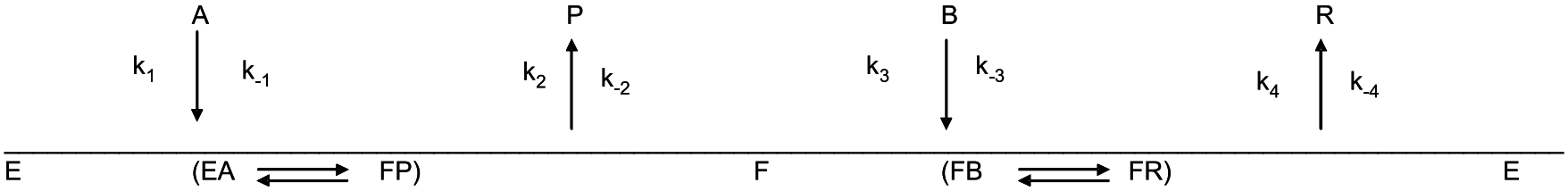
Plot of the NADH-Q2 reductase reaction with varying Q concentrations and fixed concentrations of NADH.
Lineweaver-Burk plot (*inset*), slopes (Vmax/Km) of the lines are not affected by NADH concentration—ping pong mechanism



K_m^{NADH} = 42 uM, K_m^{Q2}= 12.5uM, V_{max}=26 unit mg^{-1}

Ping Pong tetra-uni mechanism

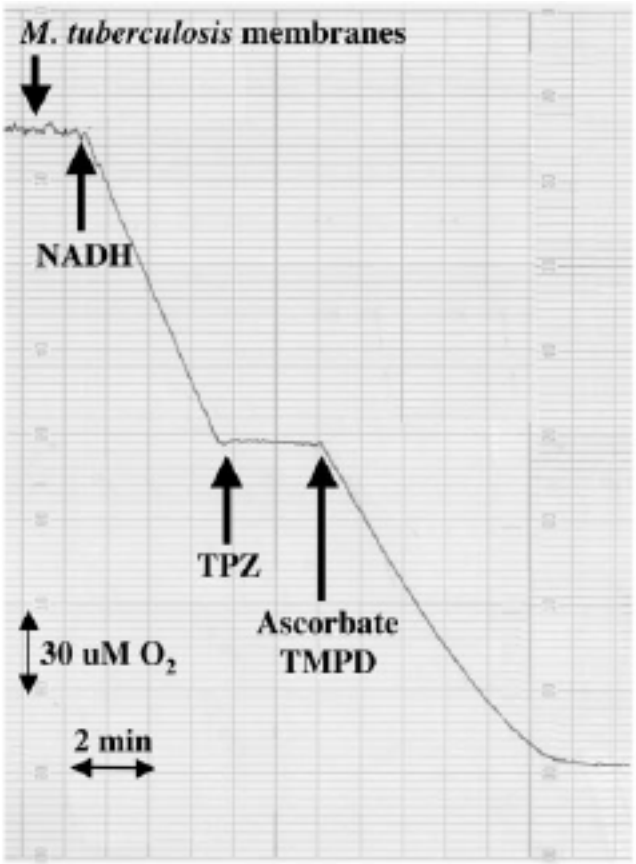
- NDH-2 catalyzes the following two electron transfer reactions:
- $Ndh(Fl_{ox}) + NADH \rightarrow Ndh(Fl_{red}) + NAD^+$ (eq1)
- $Ndh(Fl_{red}) + Q \rightarrow Ndh(Fl_{ox}) + QH2$ (eq2)



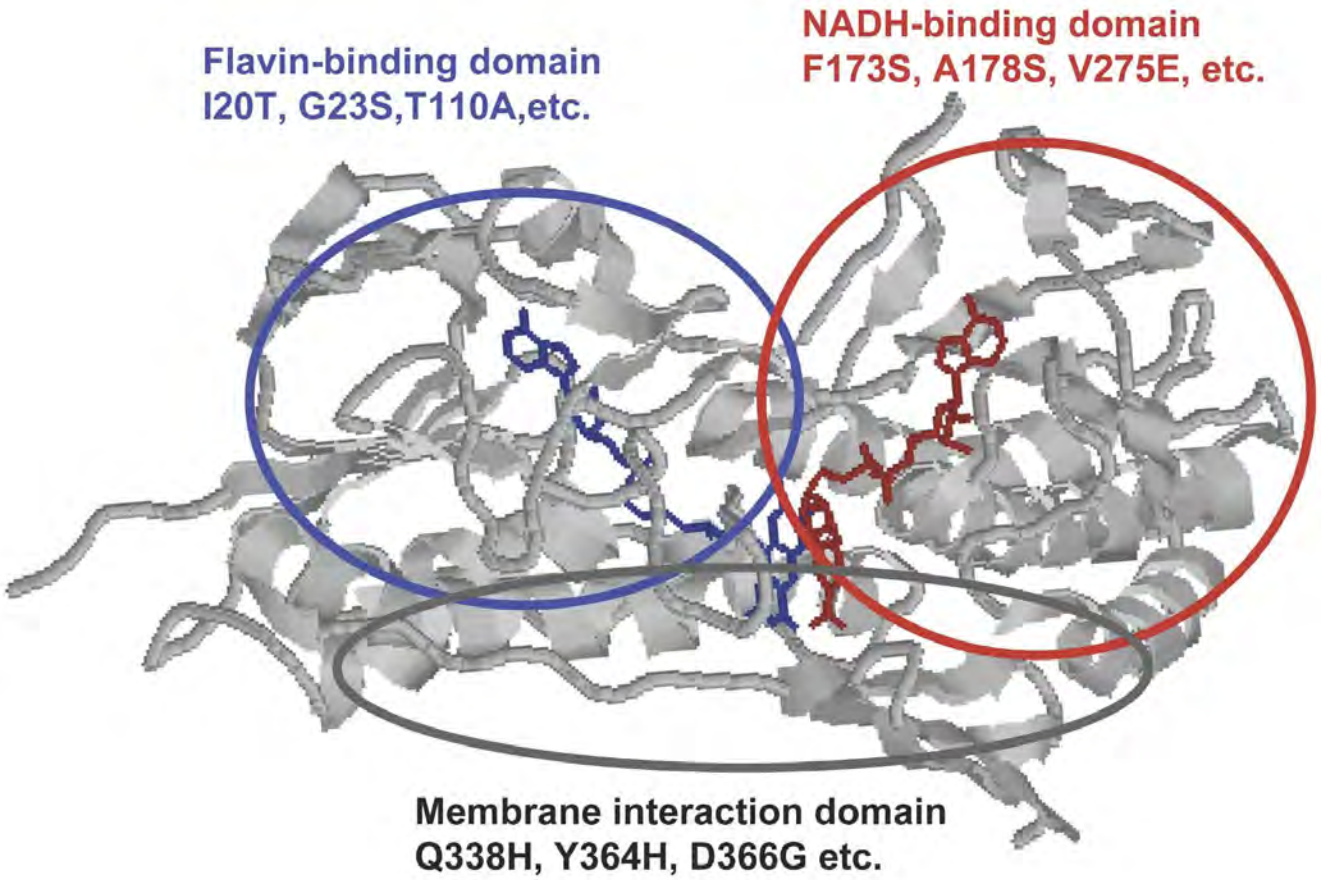
E is Mtb NDH-2, A is NADH and B is the quinone

Phenothiazine inhibition of Mtb respiration.

A



(A) TPZ inhibition of NADH-dependent oxygen consumption by Mtb membranes measured with a Clark-type oxygen electrode. Respiration was initiated by the addition of 10 mM NADH and arrested upon the addition of 1mMTPZ. Addition of 10mM ascorbate and 1 mM TMPD produced an immediate resumption of respiration.



A 3D model of *E. coli* Ndh according to Schmid and Gerloff (2004). Putative flavin-, NADH-, and membrane-binding domains are shown in ovals.

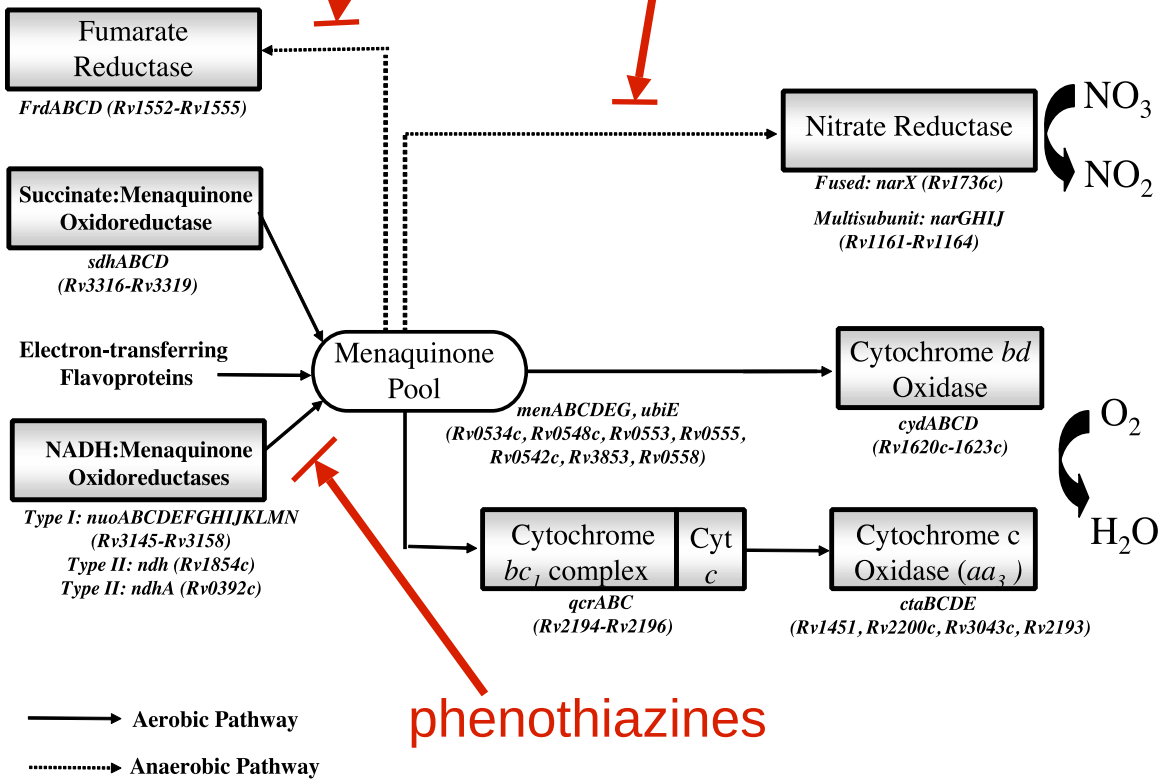
A drug for dormant TB

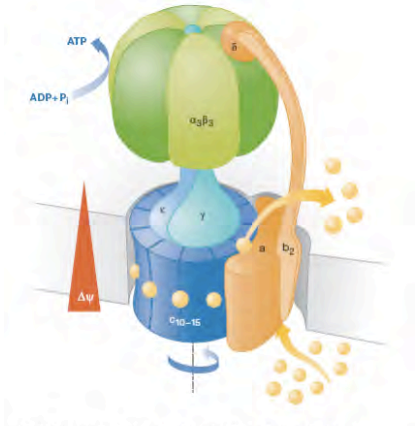
Drug	MBC(mg/L)	
	Log-phase	6-week-starved
Rifampin	<0.625	10
Trifluoperazine	10~20	40
Chorpromazine	10~20	40
Isoniazid	<0.625	80
Ethionamide	<0.625	>160
Capreomycin sulfate	0.625	>160
Amikacin sulfate	<0.625	>160
Thiacetazone	<0.625	>160
Ethambutol	0.625	>160
Streptomycin sulfate	<0.625	>160
p-aminosalicylic acid	<0.625	>160
Ofloxacin	<0.625	>160
Tetracycline	10~20	>160
Cycloserine	10~20	>160
Erythromycin	40	>160
Dapsone	>40	>160

MBC_{99}s of 17 Drugs for Log-phase and 6-week-starved *M.tuberculosis*H37Rv by cfu counts.

We shall destroy the respiratory chain by recognizing the heterogeneity of its parts!

diarylquinolines





phenothiazines

We shall go on to the end, we shall fight in France, **we shall fight** on the seas and oceans, **we shall fight** with growing confidence and growing strength in the air, we shall defend our Island, whatever the cost may be, **we shall fight on the beaches, we shall fight** on the landing grounds, **we shall fight** in the fields and in the streets, **we shall fight** in the hills; **we shall never surrender.**

WSC June 4, 1940

Can molecular computing say anything

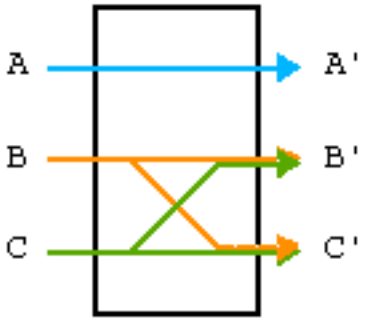
based on irreversible nature of computation

The Fundamental Physical Limits of Computation
What constraints govern the physical process of computing? Is a minimum amount of energy required, for example, per logic step? There seems to be no minimum., but some other questions are open
by Charles H. Bennett and Rolf Landauer
Scientific American **253**(1):48-56 (July, 1985).

A Fredkin Gate: Logically reversible with no energy limit on the computation

A.

A	B	C	->	A'	B'	C'
1	1	1		1	1	1
1	1	0		1	0	1
1	0	1		1	1	0
1	0	0		1	0	0
0	1	1		0	1	1
0	1	0		0	1	0
0	0	1		0	0	1
0	0	0		0	0	0



CAB is a piece of DNA that we can synthesize

a NAND gate

A.

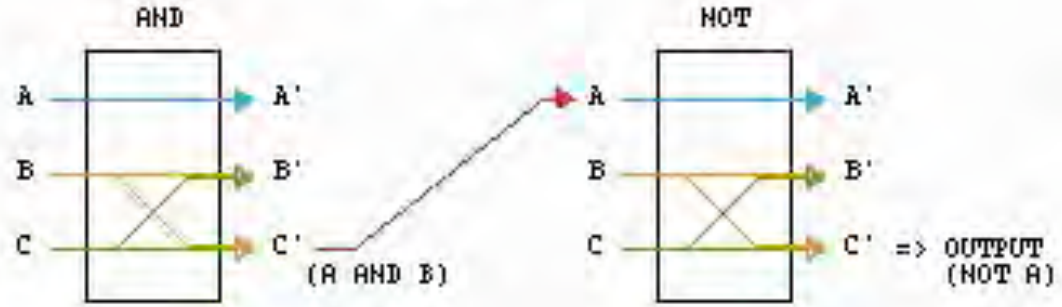
AND

A	B	C	->	A'	B'	C'
1	1	0		1	0	1
1	0	0		1	0	0
0	1	0		0	1	0
0	0	0		0	0	0

NOT

A	B	C	->	A'	B'	C'
1	0	1		1	1	0
0	0	1		0	0	1

NAND gate



B.

1 A 0--C-> => 1 A 0--C'A'B'

<-C'A'B'

1->

1 A 0--C'A'B' => 1 A 0

<-0

Figure 2

Why reversible?

Minimal energy expense

Detection and correction of intrusion

Error checking by reversing computation to recreate inputs

Bidirectional debugging

In principle it can take minimal energy to go through a biochemical gate

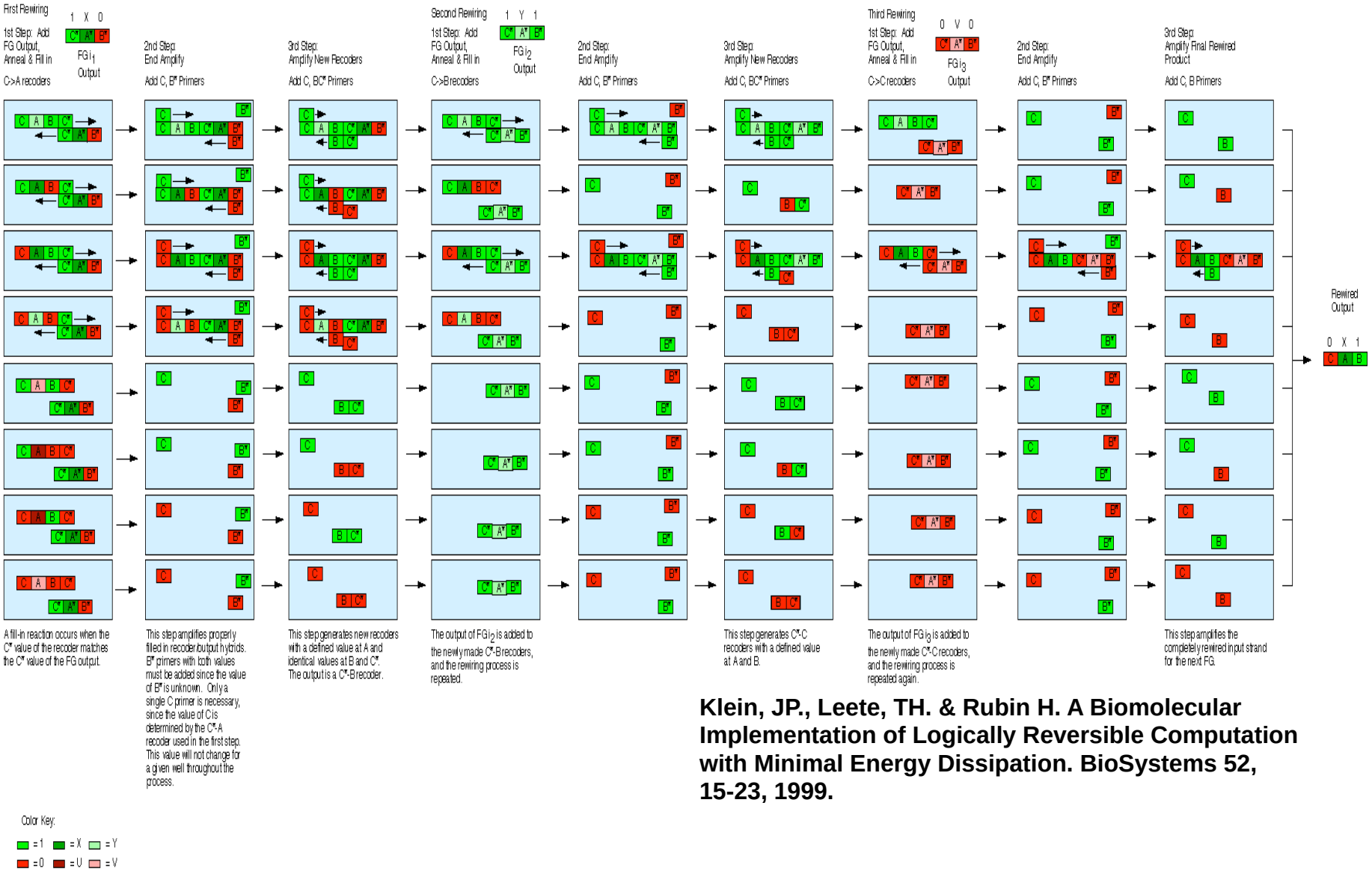
$DNA_n + dNTP \Longleftrightarrow DNA_{n+1} + PPi$

$\Delta G = kt \ln[dNTP/PPi]$

If dNTPs are just 1% over the equilibrium value:
$\Delta G = kt \ln[10.1/10]$ or about 0.01kT

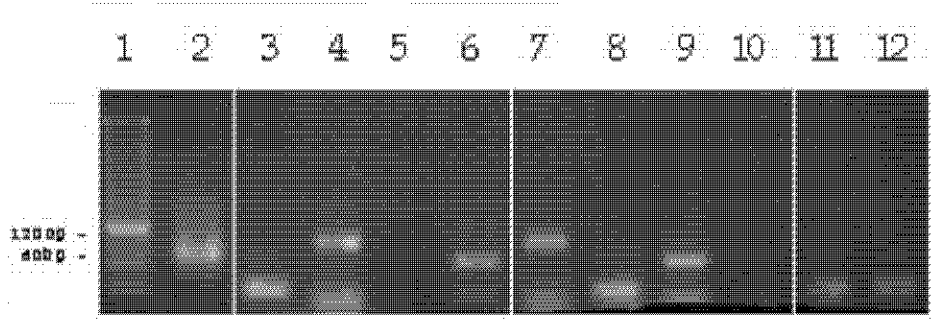
a modification of an idea in Bennett and Landaur's Sci. Am paper—suggested using RNA

We synthsized the oligonucleotides and ran the reactions



Klein, JP., Leete, TH. & Rubin H. A Biomolecular Implementation of Logically Reversible Computation with Minimal Energy Dissipation. BioSystems 52, 15-23, 1999.

The gate works in the lab



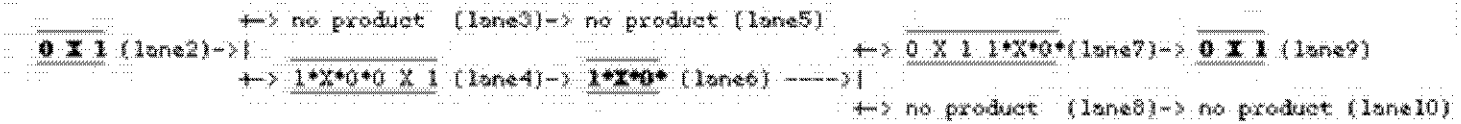


Figure 3

How fast could one go through one gate?

$t_{1/2}$ annealing:	3 sec.
DNA polymerization rate:	15 bases/sec
For 60 bases pair input:	10 sec

Some very hard problems in nature (biology-biochemistry) "solved" using physical algorithms that reduce the hardness

"Problems"	*"Algorithms"*
Search optimization	Cooperativity
Hill climbing—energy reduction	Heterogeneity
Allocation of resources	Stochasticity
Self assembly	
Reversible computation	
Satisfiability	
Controllers for nanomachines	
add your favorite problem	

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